Report for 2005ME43B: A Pilot Study to Evaluate the Potential for River Water Toxicity to Increase Following Dam Removal

Publications

- Articles in Refereed Scientific Journals:
  - Elskus, A.A., LeBlanc, L.A., Kim, C., Van Beneden, R., Mayer, G. (2006) Studying toxicity: The effects of dam removal. International Water Power and Dam Construction March 2006: 20-32.

Report Follows

A Pilot Study to Evaluate the Potential for River Water Toxicity to Increase Following Dam Removal

Adria A. Elskus[1], Lawrence LeBlanc[2], Gregory Mayer[3], Carol Kim[3], Rebecca van Beneden[2,3] and L. Brian Perkins[4]

[1]U.S. Geological Survey, Maine Field Office, Dept. of Biological Sciences, University of Maine, Orono, ME 04469
[2]School of Marine Sciences, University of Maine, Orono, ME
[3]Biochemistry, Microbiology and Molecular Biology Dept, University of Maine, Orono, ME
[4]Dept of Food Science and Human Nutrition, University of Maine, Orono, ME

Abstract

Precipitous declines in Atlantic salmon (*Salmo salar*) populations have led to the loss of all wild salmon in the United States except Maine where Penobscot River populations have the greatest restoration potential. Dam removal has been identified as the most important restoration strategy, but this can result in the release of contaminants from sediments into overlying waters. To evaluate the toxic potential of Penobscot River sediments, we used a simple laboratory-based, sediment resuspension design and two well-established aquatic toxicology models, fathead minnows (*Pimephales promelas*) and zebrafish (*Danio rerio*). Sediments were collected from Penobscot River sites downstream of the two dams slated for removal and characterized for resuspension-mediated chemical desorption and biological toxicity. Sediment resuspension elevated Penobscot river water concentrations of organic pollutants (PAHs) and several heavy metals, including mercury. Fathead minnow embryo development was delayed in fish exposed to resuspension water, but embryo-larval survival, hatch success, and the incidence of developmental abnormalities were unaffected. Innate immune response was significantly weaker in zebrafish embryos exposed to resuspension water. CYP1A1 gene expression was elevated up to 70 fold in zebrafish embryos exposed to river water, with or without sediment-suspension. There was no evidence of estrogen-active substances in any of the treatments as measured by vitellogenin production by zebrafish embryos and estrogen-sensitive mammalian MVLN cells. Zebrafish carrying a transgene for metal-responsive elements did not respond to any water treatments. Together these results demonstrate that resuspension can release biologically active chemicals from Penobscot River sediments. Studies of resident Penobscot River species, including Atlantic salmon, are needed to evaluate if the biological effects we observed reflect general fish responses to sediment resuspension.

Problem and Research Objectives

There has been a precipitous decline in Atlantic salmon (*Salmo salar*) populations in North America, resulting in extirpation of this species in the US except for 8 Maine rivers whose populations were listed as endangered in 2000. Dam removal has been identified as the most important strategy for restoring salmon populations in Maine (NationalResearchCouncil 2004) and two dams on Penobscot River, Great Works Dam

and Veazie Dam, have been designated for removal. Dam removal can result in release of contaminants from riverine sediments into overlying waters, potentially increasing water toxicity to resident species, including anadromous fish. Because dams will be removed as part of the Penobscot River Restoration Project, there is a need to evaluate the toxic potential of Penobscot River sediments prior to dam removal.

Dam removal in rivers leads to resuspension of sediments, due both to release of sediments behind dams and resuspension of sediments deposited elsewhere in the river. Sediments act as repositories for persistent organic pollutants, including organochlorines, aromatic hydrocarbons, organo-metals and pesticides. Sediment resuspension can release these chemicals to overlying waters (Simpson, Apte and Batley 1998; Latimer, Davis and Keith 1999; Bogdan, Budd, Eadie and Hornbuckle 2002; Martino, Turner, Nimmo and Millward 2002; Hornbuckle, Smith, Miller, Eadie and Lansing 2004), leading to changes in their physico-chemical properties, including potential alterations in toxicity. Aquatic organisms are particularly vulnerable to dam removal, being subjected to the multiple stressors associated with a habitat undergoing dramatic physical, chemical and biological changes (Francisco 2004).

The goal of the proposed study was to determine if resuspension of sediments following dam removal will significantly increase the toxicity of riverine water to early life stage fishes. Physiological responses, including immune function, reproductive success, and embryologic development, are predictive of population level effects and are commonly used as indicators of contaminant stress. The use of transgenic fish that incorporate reporter genes indicative of exposure to metals provide powerful tools for identifying the potential biochemical mechanisms underlying these effects. Understanding underlying mechanisms provides predictive capabilities for extrapolating results to other species.

We used a simple laboratory-based, sediment resuspension design and two well-established aquatic toxicology models, fathead minnows (*Pimephales promelas*) and zebrafish (*Danio rerio*), to evaluate if resuspension of Penobscot River sediment significantly elevates the toxicity of river water as measured by fish survival, hatch success, development, and immune competence, whether bioactive metals and/or endocrine disrupting substances are present, and to provide preliminary information on the types of chemicals likely to desorb during resuspension.

Hypothesis: Release of contaminants bound to Penobscot River sediments during resuspension events will significantly increase the toxicity of river water to early life stage fishes.

Objectives:

- Use a simple laboratory model to simulate resuspension and chemical desorption of Penobscot River sediments
- Quantify persistent organic contaminants released by resuspended sediments
- Use fish models to evaluate lethality and early life stage effects of river water before and after sediment resuspension.
- Use transgenic zebrafish to detect the presence of bioactive metals in river water

- Develop recommendations for management of Penobscot River sediments prior to dam removal

Methodology

Sediment and water collection.
Sediments were collected in August 2005 from two Penobscot River sites, Bowdoin Point and the Marsh River region of Frankfort Flats. These sites, downstream of the former Holtrachem chemical plant and the Veazie dam, contain fine-grained sediments known to have elevated levels of mercury (K. Merritt, pers comm). A composite of three grab samples of the top 5 cm of sediment was taken at each site, homogenized, aliquoted and stored in pre-cleaned, acid-rinsed Teflon bottles at -20 C. River water was collected using pre-cleaned 4-L amber glass bottles, and stored at 4C until resuspended (see 'Desorption' below).

A third sample from Frankfort Flats was collected from the same area as our Marsh River sample in Spring 2003 by K. Merritt (UMaine, Engineering Program), characterized for mercury at that time and stored at -20 C. We used aliquots of this homogenate (Marsh River 03) to determine if any change had occurred, as indicated by the profile and amount of chemicals released during resuspension, during this 2.5 year interval.

Desorption of particle-bound contaminants into overlying waters.
Chemical desorption of sediments was achieved using a simple laboratory method. Wet sediment (equivalent to 20 g dry weight) was weighed into 2L Teflon screw-cap jars and filled with river water. The ratio of dry sediment weight:water (10g:L) was used to represent a maximum sediment loading, as is found during bedload transport (Ogston, Cacchione, Sternberg and Kineke 2000; Campbell, Laycak, Hoppes, Tran and Shi 2005). Pre-cleaned Teflon-coated stir bars were added to each jar, and sediments were stirred continuously on stir plates under refrigeration (4°C) for approximately 72 hours. This timeframe was chosen because in several studies of desorption of semi-volatile hydrophobic contaminants from sediments, maximum desorption of an initial fast-desorbing sorbed phase has been found to occur on the order of hours to a few days (Karickhoff and Morris 1985; Jepsen, Borglin, Lick and Swackhammer 1995; Lick and Rapaka 1996).

After stirring, sediments were allowed to settle for at least 48 hours. Samples were then filtered through pre-combusted glass fiber filters (Whatman GF/F, Maidstone, England), using a Buchner funnel and a vacuum aspirator flask. The filtrate was split into three aliquots for metals, organics, and biological assays and stored in pre-cleaned, acid-rinsed Teflon jars under refrigeration (4°C) until used for biological assays and/or chemical analysis.

Chemical analysis of Penobscot river water and 'resuspension water'.
River water and 'resuspension water' were characterized for general water quality (Table 1), and screened for a variety of organic contaminants, including priority polynuclear

aromatic hydrocarbon (PAH) pollutants, polychlorinated biphenyl (PCB) congeners, organochlorine pesticides, and metals.

Organic analysis

Two methods of organics extraction were utilized and compared using analyte recoveries from matrix spiking experiments. The first method was adapted from Westbom et al. (Westbom, Thorneby, Zorita, Mathiasson and Bjorklund 2004) using C18-impregnated Empore discs (3M Corporation, St. Paul, Mn). One-liter water samples previously filtered through glass fiber filters were extracted through Empore discs using a standard 47 mm Millipore filtration apparatus (Millipore Corporation, Billerica, MA). Prior to extraction, 2000 ng of the PAH surrogate (ortho-terphenyl) and the PCB surrogate (CB-143) standards (dissolved in methanol) were added to the sample to give a final concentration of 2 ng/uL. The discs were allowed to dry for approximately 2 hours in the hood, and then frozen (at -20°C) until analysis.

Samples were eluted from the Empore discs no later than 3 days after extraction. Empore discs were eluted with 3 x 5 mL of methylene chloride, as described (Westbom, Thorneby, Zorita, Mathiasson and Bjorklund 2004). These extracts were then dried over sodium sulfate and reduced in volume to 500 uL using a Zymark Turbovap II sample concentrator (Zymark Corporation, Hopkinton, MA). Internal standard (d_{10}-phenanthrene) was then added and the sample brought to a final volume of 500 uL via evaporation with a stream of nitrogen.

The second method of organic extraction was the more traditional liquid-liquid extraction method. One liter of filtered resuspension water was placed in a 2L separatory funnel and extracted with 3 x 100 mL of methylene chloride. Extracts were combined, dried over sodium sulfate and reduced in volume using a Buchi Rotavor rotary evaporator (Buchi Labortechnik AG, Flawil, Switzerland). Internal standard was added and the final sample volume of 500 uL was achieved via evaporation with a stream of nitrogen.

Instrumental Analysis- Organics

Target analytes included 19 PCB congeners, DDT metabolites and 17 EPA priority pollutant PAHs (Table 2). These compounds were chosen as a preliminary screen targeting the most ubiquitous aquatic pollutants as well as those identified as priority pollutants by the EPA. Samples were quantified from the internal standard added immediately prior to instrumental analysis (d_{10}-phenanthrene) and corrected for surrogate recoveries (ortho-terphenyl for PAH analytes, PCB congener 143 for PCBs and DDT metabolites).

Samples were analyzed on a Hewlett Packard 6000 GC interfaced with a Hewlett-Packard 7200 mass spectrometry system (Agilent Corporation, Palo Alto, CA). Instrumental conditions are listed in Table 3. The instrument was run in selected ion monitoring (SIM) mode, which provided maximum sensitivity for the ions of interest. A five-point calibration curve was analyzed prior to every run.

Instrumental Analysis- Metals
Filtrate was characterized for total mercury by cold-vapor atomic fluorescence (E.P.A. 2002), and for 14 additional metals by high resolution inductively coupled plasma mass spectrometry (HR-ICP-MS) using a Thermo-Electron Element2 essentially as described (Osterberg, Handley, Sneed, Mayewski and Kreutz 2006) by the Sawyer Environmental Research Laboratory (UMaine). Isotopes were measured in low and medium resolutions; ^{107}Ag, ^{111}Cd, ^{133}Cs, ^{138}Ba, ^{208}Pb, ^{238}U were measured in low resolution and ^{27}Al, ^{51}V, ^{52}Cr, ^{55}Mn, ^{59}Co, ^{60}Ni ^{63}Cu, ^{66}Zn were measured in medium resolution. An ApexQ sample introduction system was used with a 200µL/min self aspirating nebulizer. Method detection limits for total mercury were 0.04 ng/L (MDL) and 0.2 ng/L (MDL adjusted for dilution). Detection limits (ng/L) for the additional 14 metals were: Cd 0.03, Cs 0.007, Ba 0.33, Pb 0.15, U 0.006, Ag <10, Al 0.05, V 0.07, Cr 0.14, Mn 0.84, Co 0.41, Ni<10, Zn 10, and Cu 3.5 ng/L.

Quality Assurance and Quality Control- Chemical Analyses
Organics: Prior to sample analysis, matrix spikes, which were river water samples spiked with the analytes of interest, were processed to compare recoveries from the two extraction methods above. We found recoveries to be lower and much more variable using the Empore disc method when compared to liquid-liquid extractions (Tables 4,5) and so decided to extract the filtered "resuspension water" samples using liquid-liquid extraction. Duplicate samples from each site were analyzed, along with two procedural blanks, which consisted of 1L of filtered, deionized water carried through the entire liquid-liquid extraction procedure.

Average recovery of the ortho terphenyl surrogate was 75 $\pm$ 17 % (mean $\pm$ standard deviation) and was 96 $\pm$ 24 % for the chlorobiphenyl 143. Procedural blanks revealed no detectable PCBs, DDTs or PAHs.

Metals: A full suite of quality assurance/quality control samples were run for metals analysis in accordance with the methods used (E.P.A. 2002; Osterberg, Handley, Sneed, Mayewski and Kreutz 2006), including filter blanks, calibration standards, standard reference materials and matrix spike recovery samples. Some of the QA/QC results for metals appear in Tables 7 and 8.

Sources and maintenance of fathead minnows and zebrafish
Fathead minnows (U.S. Environmental Protection Agency, Cincinnati, OH) and zebrafish (UMaine Zebrafish Core) were used as first-step, inexpensive screening tools to determine if resuspension of sediments significantly elevates river water toxicity to fish. Fathead minnows were housed and spawned at the Aquaculture Research Center, University of Maine, in flow-through tanks using standard procedures (EPA 1987; ASTM 1992). Water quality conditions in these tanks are listed in Table 6. Zebrafish embryos (AB strain) and adults were provided by the UM Zebrafish Core Facility.

Fathead minnow spawning
Reproductively mature fathead minnows were held in 25 gallon, flow-through community tanks at 25 C on a 16/8 L/D cycle, females in one tank, males in another tank.

To obtain embryos, one male was placed into a tank containing 6-8 females. Curved, PVC tiles were placed in each tank and checked daily for embryos. When found, the tile was removed to a separate tank, set against an airstone, and the embryos left to water-harden (3-4 hours) prior to removal by gently rolling them off into a Petri dish containing culture water. Live, healthy embryos of the same developmental stage were selected from the spawn and placed into exposure chambers (beakers or vials, see above). A typical spawn yielded ~ 200-300 embryos per female. A separate spawn was used for each replicate experiment.

Fathead minnow and zebrafish embryo exposures

FHM and ZF embryos were exposed to culture water, river water or 'resuspension water' in static renewal exposure systems for 5 (zebrafish) to 10 (fathead minnows) days. Preliminary studies were run to determine the time course of development from Stage 0 – larval stage 2 (L2) (Devlin, Brammer, Puyear and McKim 1996), to evaluate and identify contaminant-induced developmental abnormalities, and to identify optimal conditions for fathead exposures. For the preliminary studies, fathead embryos were placed in PVC cups with mesh bottoms placed inside 250 mL beakers (25 embryos/125 mL/beaker x 3 beakers) for 12 days. For the Penobscot River experiments, fathead embryos were exposed in 20 mL vials (5 embryos/vial, 5 vials per replicate). Fatheads were held under 16/8 L/D at 25 C for 10 days.

For the respiratory burst assays, zebrafish embryos were exposed to test water at 28C, 14/10 L/D from fertilization through day 5 in 100 mm diameter Petri dishes. All embryos were examined daily and dead individuals removed. For fathead minnow and zebrafish experiments, one spawn was used for each replicate experiment (one spawn=one replicate).

For the estrogen and metal bioassays, zebrafish embryos and MVLN cells were exposed to one of five treatments: distilled water with sodium bicarbonate and sea salt with a constant conductivity of 800μS and pH 6.87 (laboratory water control), Penobscot River water (river water), or 'resuspension water' from one of the three sites. The distilled water with sodium bicarbonate and sea salt is the water in which all zebrafish are maintained at the University of Maine Zebrafish Facility.

MVLN cell exposure conditions

MVLN cells were exposed to the same treatments as described above for zebrafish embryos except that all water samples were sterile filtered, diluted 1:5 or 1:10 in cell media (described below) and the sodium bicarbonate water was replaced with sterile distilled water.

Fathead minnow development: Hatch Success, Mortality and Developmental abnormalities

Mortality, developmental stage, developmental abnormalities, hatch success and days to reach L2 stage were recorded daily. Embryos were examined microscopically for evidence of hatching, death, developmental stage and abnormalities (e.g. tube heart, yolk-sac edema, pericardial edema, skeletal defects). For the Penobscot River studies,

embryos were exposed from developmental stage 13 (set #1, conducted in March/April using one male and 5-6 females) or developmental stage 11 (set#2, conducted in May 2006 using a new male with 6-8 new females), through L2.

Dose-response β–naphthoflavone experiment
A preliminary dose-response study was conducted using a well-studied PAH toxicant, β–naphthoflavone (βNF), to identify developmental abnormalities and to evaluate the effects of a PAH compound similar to those we would expect to find in contaminated river sediments. We also used this experiment to optimize exposure conditions for the Penobscot River experiments. For the βNF study, embryos were aqueously exposed to culture water, vehicle control (0.001% DMSO in culture water), or to a range of βNF doses (0.0001 – 1.0 μM) from developmental stage 13 (7-8 h post-fertilization) through larval stage L2, a stage unambiguously identified by the presence of an inflated swim bladder (Devlin, Brammer, Puyear and McKim 1996). β–naphthoflavone dosing solutions were prepared in culture water by serial dilution of a stock solution (10 μM βNF dissolved in DMSO).

Respiratory burst assay
The respiratory burst of white blood cells, specificially granulocytes, monocytes, and macrophages, in response to a pathogen is a measure of the strength of the innate immune system in an organism. The respiratory burst involves the reduction of molecular oxygen to the anionic radical, superoxide, which can be converted into a number of different reactive oxygen species (ROS). A strong innate immune system reacts to pathogens with a strong respiratory burst. Zebrafish embryos were exposed to river water, 'resuspension water' or laboratory contol water (described above) through 5 days post-fertilization, then transferred to a 96-well plate (one embryo per well), and analyzed for respiratory burst as described (Hermann, Millard, Blake and Kim 2004). Five replicate experiments were conducted, using a different spawn for each replicate. One 96 well plate was run for each replicate, for a total of 5 plates. Additional experiments were run to optimize conditions, these are not reported here.

Zebrafish Embryo Exposures for gene expression endpoints
Zebrafish embryos were exposed to 30mL control laboratory water, river water and ‘resuspension water’ treatments in polystyrene Petri dishes beginning at the 1-cell stage. Embryos that were exposed to distilled water with sodium bicarbonate/sea salt (control laboratory water), river water or ‘resuspension water’. Treatment water was changed every 24 hours. Embryos were exposed for 48-168 hours. At each time point, 25 embryos were pooled per treatment and placed in 200μL lysis buffer. RNA was isolated in triplicate from each pool at each time point and extracted according to Ambion RNAqueous protocol. RNA integrity and concentrations were determined by Agilent RNA Nano 6000 chips.

Zebrafish CYP1A1 and VG mRNA Expression
CYP1A1 primers were designed using Primer3 based upon cDNA sequences attained from GenBank. CYP1A1, vitellogenin, and 18S primer sequences are provided in Table 7. CYP1A1 and vitellogenin expression were determined by fluorescence-based

quantitative real-time polymerase chain reaction (qRT-PCR) using the BioRad iScript SybrGreen qRT-PCR kit and Statagene MX4000. Expression fold change compared to embryos exposed to control laboratory water was determined according to Livak and Schmittgen (Livak and Schmittgen 2001).

Transgenic Zebrafish Embryo Exposure

A transgenic zebrafish line (ZM9) carrying a green fluorescent protein (GFP) reporter driven by the zebrafish metallothionein (MT) promoter was used to detect the presence of metal-active compounds in the treatment waters. Transgenic embryos were exposed to 30mL control and 'resuspension water' treatments in polystyrene Petri dishes beginning at the 1-cell stage for 96 hours. Ninety-six hours had been previously determined as the optimum stage at which to measure fluorescence (data not shown). Fluorescence was determined by a Packard Fusion platereader. Two replicate wells were run per experiment and each experiment was replicated three times (n=3).

MVLN cells

MVLN cells are MCF-7 human breast cancer cells transfected with a luciferase reporter gene downstream of the *Xenopus laevis* vitellogenin promoter. This cell line was developed by Dr. Michael Pons and graciously donated to us by Dr. John P. Giesy. Cells were maintained in 1:1 DMEM and Ham's F-12 media with phenol red and exposed to treatments in 1:1 DMEM and Ham's F-12 media without phenol red to reduce estrogenic interference. The vitellogenin promoter region is characterized by four estrogen responsive elements and exposure to estrogenic compounds provokes luciferase activity with a resulting increase in luminescence. By measuring luminescence after cell exposure, relative vitellogenin expression can be determined. To ensure that estrogen responsive elements were activated by estrogen agonists, the estrogen receptor antagonist, ICI, was used as a negative control. If a chemical is exerting estrogenic activity via the estrogen receptor, co-treatment with ICI will specifically block this effect, thereby confirming the specificity of the chemical's action. Cells were maintained and exposed using sterile techniques. All treatment water was sterile filtered using 0.2µm Acrodisc syringe filters. MVLN cells were exposed to media, three doses of 17β-estradiol (positive control), river water or 'resuspension water' treatments in 96-well polystyrene microplates. Luminescence was determined using the Promega Steady-Glo Luciferase Assay system and measured by the Packard Fusion platereader. Three replicates were run for each treatment.

Statistics

Quantitative RT-PCR data was analyzed using nested one way analysis of variance (ANOVA). All statistical analysis was carried out on raw Ct values, with very stringent standards causing higher likelihood of Type II error (failing to determine significance for a data point that is actually significant). Both equal variance and normality were validated prior to ANOVA. One way ANOVA allowed for analysis between all treatments for a given gene. When statistically significant differences were found between treatment groups, Tukeys HSD was used to determine which treatments were significantly different from unexposed controls at $\alpha=0.05$ for each test. Normalizing genes (18s rRNA) were also analyzed by one way nested ANOVA and $p>0.5$ was used to validate that exposures

did not influence levels of 18s rRNA, the gene against which CYP1A and VG mRNA values are normalized. All statistical analyses were done using SYSAT 11 software (SYSTAT Inc.).

For the fathead minnow development experiments (hatch success, percent survival, percent occurrence of abnormalities) and for the zebrafish respiratory burst experiments, differences among treatments were evaluated by single-factor ANOVA. To statistically evaluate development time (days to reach L2stage), we used 2-way ANOVA (treatment x day). We used a significance level of $P<0.05$ throughout.

Summary of Principle Findings

- Resuspension, even under conditions of high sediment:water ratios for extended periods of time under vigorous stirring, resulted in relatively low contaminant concentrations (ppt) in the river water. This could be due to low levels of contaminants in the sediments, or low contaminant release. To differentiate these possibilities requires measurement of contaminant concentrations in the test sediments, which was too costly to include in this initial pilot study.

- Repeated resuspension of Frankfort flats sediments does not appear to have affected the release of organics or metals; sediments collected from Marsh River in 2003 and 2005 showed no difference in chemical profile or concentration in resuspension water.

- Sediment resuspension provoked delays in fathead minnow development and suppressed the innate immune system of zebrafish. Effects on development were slight, and likely not biologically significant. Suppression of the innate immune response should be followed up with additional studies using fish species resident in the Penobscot River, such as fathead minnows, Atlantic salmon and smallmouth bass.

- Penobscot River water alone elevated gene expression of the pollutant biomarker, CYP1A1, in zebrafish embryos, suggesting that chemical inducers are likely present in the river. Although there was a tendency for sediment resuspension to further elevate CYP1A1 expression, this effect was not statistically significant. These studies should be repeated with other fish species, particularly those resident in the Penobscot river, to determine whether immune suppression is a widespread fish response to sediment resuspension. Further analyses to identify the chemical inducers of CYP1A1 in this system could be used to identify sources and chemicals not yet known in this watershed.

- We found no evidence of endocrine disruption, suggesting that resuspension of sediments from these particular sites is unlikely to provoke adverse estrogenic effects in Penobscot River fish.

Conclusions & Significance

- The multi-pronged approach of this study, chemical analysis coupled with multi-level biological effects, provides a powerful assessment of potential risk useful for any resuspension event, including spring and fall floods, dredging, stream bank remediation (riparian removal) or any event that resuspends significant amounts of sediment in the environment.

- Sediment-bound chemicals from the Frankfort Flats region appear to be fairly recalcitrant to release as evidenced by the finding that repeated tidal and spring/fall flood-mediated resuspension does not appear to reduce sediment release of contaminants over time, including release of mercury. Dam removal in the Penobscot River is not likely to change this.

- Resupsension of Frankfort Flats sediments following dam removal is not likely to be a significant source of contaminants and will likely produce little, if any, adverse biological effects due to released contaminants.

- Whether sediment movement following dam removal will have physical, non-contaminant effects (e.g. smothering) requires further study.

Recommendations

Based on these preliminary findings, we find no reason to recommend management of Frankfort Flats sediments prior to removal of the Veazie and Great Works dams.

Studies of chemical release and biological toxicity should be conducted on other sediments downstream of these dams, particularly the PAH-contaminated sediments in Dunnett's Cove in Bangor (Gagnon 2004), prior to removal of the Veazie and Great Works dams.

Detailed Results and Discussion

This study addresses the relative importance of sediment resuspension as a source of toxicants, provides preliminary information on which chemicals are desorbed and provides recommendations as to whether river sediments should be remediated or removed prior to dam removal.

Chemical release with sediment resuspension.

a. Metals

Very low concentrations of metals were observed in Penobscot River water alone. Resuspension appeared to elevate total mercury levels (Table 8), and the concentrations of some, but not all, of the 14 other metals analyzed (Tables 9A, 9B). Because this was a pilot study with limited funds, only one sample was analyzed for each site, precluding

statistical analysis. However, differences among water samples are slight and likely statistically insignificant.

b. Organics

As for the metals, very low concentrations of organic compounds were observed in Penobscot River water alone. Of the 17 priority PAH pollutants analyzed for, only 5 PAHs were detected. Resuspension of Penobscot river sediments elevated levels of these PAHs by 2-50 fold relative to river water alone (Table 10). Of the 209 possible PCB congeners, none were detected. Similarly, the chlorinated pesticides DDT, DDE and DDD, were not detected.

Enhanced release of hydrophobic contaminants from suspended sediments have been demonstrated in a number of studies for organic contaminants (Latimer, Davis and Keith 1999; LeBlanc, Gulnick, Brownawell and Taylor 2006) as well as metals (Cantwell, Burgess and Kester 2002), including mercury (Kim, Mason, Porter and Soulen 2004). The phase associations of these released contaminants range from particulate, colloidally-associated to truly dissolved, depending on a variety of factors, including pH and the degree of oxidation of sediment mineral phases (Eggleton and Thomas 2004). Resistance to desorption has been described in a number of studies (Chen and Mayer 1999; Lamoureux and Brownawell 1999) and so it is possible that PAH compounds present in these sediments are resistant to desorption. The presence of soot carbon has been shown to retard PAH desorption from sediments in a number of studies (see (Gustafsson, Haghseta, Chan, Macfarlane and Gschwend 1997). Also it has been shown that PAHs in highly weathered sediments are resistant to desorption (LeBlanc 2001). Examining desorption kinetics and understanding rate limitations to desorption requires chemical analysis of bioavailable, labile organics present in the sediments themselves, and was beyond the scope of this study.

The aqueous concentrations of total unsubstituted PAH we observed following resuspension of Penobscot sediments into river water (101 – 232 ppt) are in the same range as dissolved concentrations found in moderately impacted coastal areas, such as the Rhone Delta in the Meditteranean Sea (Bouloubassi and Saliot 1991). In contrast, total dissolved PAH concentrations in the heavily impacted Yangtze River in China ranged from 22 – 380 ppm (Huang, Zhang and Yu 2003) while remote lakes in the Pyrenees and Alps had extremely low PAH concentrations (0.7 – 1.1 ppt) (Vilanova, Fernandez, Martinez and Grimalt 2001). The presence of alkylated naphthalene, the only target PAH with an alkyl substituent present in our water samples, suggests that some of the PAH loading may be derived from petroleum sources (NationalResearchCouncil 1985), although more in depth characterization of the PAH distribution would be needed to demonstrate this conclusively.

One conclusion that can be drawn from the organics and metals data is that repeated resuspension of Frankfort flats sediments does not appear to have affected chemical release. Sediments collected from Marsh River in 2003 and 2005 showed no difference in chemical profile or concentration in resuspension water.

Effects in fish:
Overview
We found that resuspension of sediments from Marsh River (Marsh River and Marsh River 03) and Bowdoin Point into Penobscot River water provoked little to no toxic effects on our test organisms.

Specific findings

a. Preliminary study: Fathead minnow dose-response to β–naphthoflavone
As expected, fathead minnow embryo-larval development was adversely affected by exposure to 1 μM, the highest concentration of β–naphthoflavone we used. Exposure to this dose significantly increased the incidence of developmental deformities (Figure 1) and delayed embryo-larval development relative to controls and to all other βNF doses (Table 11). Similar deformities have been noted in other embryo-larvae exposed to PAHs in this dose range (Arzuaga 2004). Interestingly, survival and hatch success were unchanged relative to controls at any βNF dose.

b. Fathead minnow development in response to Penobscot River water exposures
Although we found a statistically significant delay in development in fathead minnow embryos exposed to 'resuspension water' relative to 'control water' treatments, this represented a delay of only 1 day, and was only observed in the March-April (Table 12), but not in the May (Table 13), experiments. The main difference between these two sets of experiments was the developmental stage of the embryos when exposures began, the May embryos being in the blastula stage (stage 11) while the March-April embryos had already reached epiboly (stage 13) when exposure began. We also found survival and percent hatch to be considerably lower in the embryos exposed at an earlier age. This may reflect age-specific differences, with the younger life-stage being more sensitive, or it may simply be that the younger embryos were not sufficiently water-hardened prior to removal from the tiles (the latter seems unlikely as the younger embryos appeared to be as resilient to physical removal as the older embryos). In any case, since all other developmental parameters were unaffected (percent hatch, percent survival, percent developmental abnormalities) relative to controls, we consider the one day delay in development to be biologically insignificant.

c. Zebrafish Respiratory Burst – Immune function
Zebrafish embryos exposed to 'resuspension water' from fertilization to 5 days post-fertilization exhibited a significantly suppressed respiratory burst response relative to embryos exposed to either culture water or river water alone (Figure 2). Suppression of innate immune response in zebrafish embryos using this assay has been reported for other toxicants, including arsenic (Hermann and Kim 2005). Suppression by arsenic was similar to that observed here, and suggests that this suppression may be biologically meaningful.

d. Endocrine disruption bioassays
We found no evidence of estrogen-active substances in Penobscot River water either before or after sediment resuspension. Several assays were used to evaluate this,

including vitellogenin gene expression and an estrogen-sensitive mammalian cell line, MVLN cells.

Vitellogenin is a phospholipoprotein whose expression is strongly elevated in oviparous animals exposed to estrogenic compounds. For example, VG levels are high in recrudescing females whose blood levels of estrogens are elevated. However, VG expression in embryos or in male fish is unexpected and inappropriate, and indicates exposure to estrogenic compounds, either through water or diet. We found very low levels of VG gene expression in zebrafish embryos and these were unaffected by exposure to any of the treatments (Figure 3). In contrast, zebrafish embryos exposed to ethinylestradiol, a potent estrogen, exhibit VG induction levels of over 40 fold above unexposed controls (data not shown). Our results suggest that either the concentration of estrogenic compounds present in river sediment released during resuspension was insufficient to provoke an estrogenic response, or that such chemicals were not present in these sediments.

The mammalian cell line, MVLN, which is sensitive to estrogenic compounds, also failed to show any significant response to Penobscot River water samples, either before or after sediment resuspension (Figure 4). This is in direct contrast to the positive control, 17β-estradiol, a potent estrogen which strongly activated the MVLN reporter, a response consistently seen with this cell line (Snyder, Villeneuve, Snyder and Giesy 2001; Furuichi, Kannan, Giesy and Masunaga 2004; Van den Belt, Berckmans, Vangenechten, Verheyen and Witters 2004). The estradiol effect was blocked by the estrogen receptor antagonist, ICI-182,780, confirming that estradiol is exerting its effects directly through the estrogen receptor.

d. CYP1A expression in zebrafish embryos

Expression of the contaminant-inducible enzyme, CYP1A, increased following exposure to Penobscot River water before (River water) and after (Bowdoin Point, Marsh River, Marsh River 03) sediment resuspension, relative to laboratory control water (Figure 5).

The time course of this induction was rapid. By 48 hours post-fertilization (hpf, the first time-point in our study), CYP1A expression was significantly increased in embryos exposed to Marsh River 'resuspension water' relative to laboratory control water (40 fold above control water), reaching peak induction at 96 hours (up to 70 fold above control water) (Figure 5). Thereafter, CYP1A mRNA levels began to fall (Figure 5). The time-course of this induction differs from that reported by others measuring CYP1A mRNA levels in zebrafish embryos exposed to the model CYP1A inducer, TCDD. In those studies, zebrafish CYP1A mRNA transcript levels were elevated in TCDD-treated embryos as early as 15 (Mattingly and Toscano 2001) and 24 hpf (Andreasen, Spitsbergen, Tanguay, Stegeman, Heideman and Peterson 2002), and continued to rise at least until 120 hpf (Andreasen, Spitsbergen, Tanguay, Stegeman, Heideman and Peterson 2002). It is likely that the rapid increase in response to TCDD reflects the high potency of this inducer relative to the compounds present in our treatment water. In contrast to our findings, however, CYP1A mRNA fold-induction was much lower than ours, being no more than 7 fold above controls (Andreasen, Spitsbergen, Tanguay, Stegeman,

Heideman and Peterson 2002). This may reflect the much shorter exposure time used by Andreasen et al (1 hour exposure) versus our exposures (168 hr).

Although the data suggest sediment resuspension further enhanced CYP1A induction, CYP1A levels in embryos exposed to 'resuspension water' were not significantly different from those in embryos exposed to river water treatments alone.

CYP1A is a biomarker of organic contaminants, including the potent toxicants PAHs, dioxins, and coplanar PCBs (Stegeman and Hahn 1994). Increased CYP1A expression indicates that at least some of these chemicals are likely present and bioavailable in Penobscot River water at levels sufficient to induce a biochemical response (Bucheli and Fent 1995). The PAH levels detected in the treatment water samples (Table 10) are approximately 10 fold lower than concentrations of a model PAH, benzo[a]pyrene, shown to induce CYP1A enzymatic activity in the gills of fish at concentrations as low as 10^{-9}M (250 ng BaP/L) (Jonsson 2003). Taken together, our results suggest that CYP1A inducing chemicals other than those we analyzed for are likely present in the treatment waters.

e. Bioassay for metal exposure

Transgenic zebrafish embryos were used as biomarkers for the presence and biological activity of metals in the water samples. The metallothionein (MT) reporter, sensitive to metals exposure, in these transgenic zebrafish failed to respond to any of the Penobscot river samples, either before or after sediment resuspension (Figure 6). Preliminary studies in our laboratory found the lowest concentrations that induce the MT transgenic zebrafish are 25 uM Zn (4,000 ng/L), 2.5 uM Cd (460 ng/L $CdCl_2$) and 25 nM Hg (4 ng/L $HgCl_2$) (Mayer, unpublished data). Cadmium concentrations in Penobscot river water and 'resuspension water' (11-34 ng Cd/L) are 10-40 fold lower than the bioactive Cd concentrations (460 ng/L), and might not be expected to activate the MT reporter in our transgenic fish. However, levels of Zn and Hg in the Penobscot river water and 'resuspension water' (9200-18,000 ng Zn /L; 3-80 ng Hg/L) are far higher than those known to induce the MT gene in our transgenic fish (4,000 ng Zn/L; 4 ng Hg/L) and would be expected to be bioactive in the transgenic fish. One possible explanation for this is loss of metals to the the plastic petri dishes used for the zebrafish exposures. Alternatively, the presence of high DOC (Table 1) may alter bioavailability of these metals to the zebrafish. Analysis of metal-spiked water as well as metal spiked, high DOC Penobscot river water, before and after storage in plastic petri dishes, is needed to determine the nature of metal dynamics in our treatment system. Based on what the transgenic assay indicates, however, we conclude that metals present in the Penobscot river water, either before or after sediment resuspension, are not likely to exert metal-mediated effects in fish.

Acknowledgements

We would like to thank Ruth Perry for construction and maintenance of the fathead minnow rearing and spawning tanks, and for conducting all aspects of the fathead

minnow developmental studies and zebrafish respiratory burst assays. Danielle Miniutti conducted the CYP1A1, VG, MVLN and transgenic studies. Akshata Nayak provided advice and assistance with the respiratory burst assays. Several students and staff at the Aquaculture Research Center provided assistance and advice, including Neil Greenberg (director), Jacqueline Hunter, Jennifer Muscato-Hansen, Soren Hansen, Erin Schramski and Dr. Sarah Lindsay. Inorganic analyses were conducted at the Sawyer Environmental Research Center by Clive Devoy and Michael Handley, Karen small measured DOC and Tanya Hyssong measured ANC and conductivity of the Penobscot river samples. Mark Nilan provided the zebrafish, Dr. Rod Bushway provided space in his laboratory for the organic analyses. Karen Merrit and Aria Amirbahman provided useful and lively discussions of metal dynamics and sediment resuspension. Supported by USGS104b Grant 01HQGR0083 through the George Mitchell Center, University of Maine.

References

Andreasen, E. A., J. M. Spitsbergen, et al. (2002). "Tissue-specific expression of AHR2, ARNT2, and CYP1A in zebrafish embryos and larvae: Effects of developmental stage and 2,3,7,8-tetrachlorodibenzo-p-dioxin exposure." Toxicological Sciences **68**(2): 403-419.

Arzuaga, X. (2004). Mechanisms of resistance to halogenated and non-halogenated AHR ligands in chronically contaminated killifish populations. University of Kentucky. Graduate Center for Toxicology. 148pp. Lexington.

ASTM (1992). "Standard guide for conducting early life-stage toxicity tests with fishes E1241-88." Annual American Society for Testing and Materials Book of Standards **11.04**: 886-913.

Ballschmitter, K. and M. Zell (1980). "Analysis of polychlorinated-biphenyls (PCB) by glass-capillary gas-chromatography-composition of technical arochlor and chlophen-PCB mixtures." Fresenius Zeitschrift Fur Analytische Chemie **22**(1): 20-31.

Bogdan, J., J. Budd, et al. (2002). "The effect of a large resuspension event in Southern Lake Michigan on the short-term cycling of organic contaminants." Journal Of Great Lakes Research **28**(3): 338-351.

Bouloubassi, J. and A. Saliot (1991). "Composition and sources of dissolved and particulate PAH in surface waters from the Rhone delta (NW Mediterranean)." Marine Pollution Bulletin **22**(12): 588-594.

Bucheli, T. D. and K. Fent (1995). "Induction of Cytochrome P450 as a Biomarker for Environmental Contamination in Aquatic Ecosystems." Critical Reviews in Environmental Science and Technology **25**(3): 201-268.

Campbell, C. G., D. T. Laycak, et al. (2005). "High concentration suspended sediment measurements using a continuous fiber optic in-stream transmissometer." Journal of Hydrology **311**: 244-253.

Cantwell, M. G., R. M. Burgess, et al. (2002). "Release and phase partitioning of metals from anoxic estuarine sediments during periods of simulated resuspension." Environmental Science and Technology **36**(24): 5328-5334.

Chen, Z. and L. M. Mayer (1999). "Sedimentary Metal bioavailability determined by the digestive constraints of marine deposit feeders: gut retention time and dissolved amino acids." Marine Ecology Progress Series **176**: 139-151.

Devlin, E. W., J. Brammer, et al. (1996). Prehatching development of the fathead minnow *Pimephales promelas* Rafinesque, U.S. Environmental Protection Agency EPA/600/R-96/079**:** 49pp.

E.P.A. (2002). "Mercury in Water by Oxidation, Purge and Trap, and Cold Vapor Atomic Fluorescence Spectroscopy." U.S. Environmental Protection Agency, Office of Water (4303), August 2002, Method 1631, Revision E, EPA-821-R-02-019.

Eggleton, J. and K. V. Thomas (2004). "A review of factors affecting the release and bioavailability of contaminants during sediment disturbance events." Environment International **30**(7): 973-980.

EPA (1987). "Guidelines for the culture of fathead minnows 'Pimephales promelas' for use in toxicity tests." EPA Report EPA/600/3-87/001.

Francisco, E. (2004). "Tales of the undammed: removing barriers doesn't automatically restore river health." Science News **165**: 235-237.

Furuichi, T., K. Kannan, et al. (2004). "Contribution of known endocrine disrupting substances to the estrogenic activity in Tama River water samples from Japan using instrumental analysis and in vitro reporter gene assay." Water Research **38**(20): 4491-4501.

Gagnon, D. (2004). "Legal Battle over Waterfront Coal Tar Deposit is Underway in Bangor, Maine." Bangor Daily News, March 17, 2004.

Gustafsson, O., F. Haghseta, et al. (1997). "Quantification of the dilute sedimentary soot phase: implications for PAH speciation and bioavailability." Environmental Science and Technology **31**(1): 203-209.

Hermann, A. C. and C. H. Kim (2005). "Effects of arsenic on zebrafish innate immune system." Marine Biotechnology **7**(5): 494-505.

Hermann, A. C., P. J. Millard, et al. (2004). "Development of a respiratory burst assay using zebrafish kidneys and embryos." J Immunol Methods **292**: 119-129.

Hornbuckle, K., G. Smith, et al. (2004). "Magnitude and origin of polychlorinated biphenyl (PCB) and dichlordiphenytricholoethane (DDT) compounds resuspended in southern Lake Michigan." Journal of Geophysical Research-Oceans **109**(C5): Art No. C05017.

Huang, J., Z. L. Zhang, et al. (2003). "Occurrence of dissolved PAHs in the Jinsha River (Panzhihua) - upper reaches of the Yangtze River, southwest China." Journal of Environmental Monitoring **5**(4): 604-609.

Jepsen, R., S. Borglin, et al. (1995). "Parameters affecting the adsorption of hexachlorobenzene to natural sediments." Environmental Toxicology and Chemistry **14**(9): 1487-1497.

Jonsson, M. (2003). A Gill Filament EROD Assay: Development and Application in Environmental Monitoring. Acta Universitatis Upsaliensis. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 920. 45pp. Uppsala. ISBN 91-554-5841-6

Karickhoff, S. W. and K. R. Morris (1985). "Sorption dynamics of hydrophobic pollutants in sediment suspensions." Environmental Toxicology and Chemistry **4**: 469-479.

Kim, E. H., R. P. Mason, et al. (2004). "The effect of resuspension on the fate of total mercury and methyl mercury in a shallow estuarine ecosystem: a mesocosm study." Marine Chemistry **86**: 121-137.

Lamoureux, E. M. and B. J. Brownawell (1999). "Chemical and biological availability of sediment-sorbed hydrophobic organic contaminants." Environmental Toxicology and Chemistry **18**(8): 1733-1741.

Latimer, J. S., W. R. Davis, et al. (1999). "Mobilization of PAHs and PCBs from in-place contaminated marine resuspension events." Estuarine, Coastal and Shelf Science **49**: 577-595.

LeBlanc, L. A. (2001). Biogeochemical controls on the persistence of saturated and aromatic hydrocarbons in urban harbor sediments. State University of New York at Stony Brook. Marine Sciences Research Centerq. 290pp. Stony Brook, NY.

LeBlanc, L. A., J. D. Gulnick, et al. (2006). "The influence of sediment resuspension on the degradation of phenanthrene in flow-through microcosms." Marine Environmental Research **61**(2): 202-223.

Lick, W. and V. Rapaka (1996). "A quantitative analysis of the dynamics of the sorption of hydrophobic organic chemicals to suspended sediments." Environmental Toxicology and Chemistry **15**(7): 1038-1048.

Livak, K. and T. Schmittgen (2001). "Analysis of Relative Gene Expression Data Using Real-Time Quantitative PCR and the 2-ΔΔCT Method." Methods **25**: 402-408.

Martino, M., A. Turner, et al. (2002). "Resuspension, reactivity and recycling of trace metals in the Mersey Estuary, UK." Marine Chemistry **77**(2-3): 171-186.

Mattingly, C. and W. Toscano (2001). "Posttranscriptional silencing of cytochrome P4501A1 (CYP1A1) during zebrafish (Danio rerio) development." Developmental Dynamics **222**(4): 645-654.

NationalResearchCouncil (1985). Oil in the Sea. Washington, DC, National Academy Press.

NationalResearchCouncil (2004). Atlantic Salmon in Maine. Washington, DC, National Academies Press.

Ogston, A. S., D. A. Cacchione, et al. (2000). "Observations of storm and river flood-driven sediment transport on the northern California continental shelf." Continental Shelf Research **20**: 2141-2162.

Osterberg, E., M. Handley, et al. (2006). "Continuous ice core melter system with discrete sampling for major ion, trace element, and stable isotope analysis." Environmental Science & Technology **40**: 3355-3361.

Simpson, S. L., S. C. Apte, et al. (1998). "Effect of short term resuspension events on trace metal speciation in polluted anoxic sediments." Environmental Science & Technology **32**: 620-625.

Snyder, S., D. Villeneuve, et al. (2001). "Identification and quantification of estrogen receptor agonists in wastewater effluents." Environmental Science & Technology **35**(18): 3620-3625.

Stegeman, J. J. and M. E. Hahn (1994). Biochemistry and molecular biology of monooxygenases: current perspectives on forms, functions, and regulation of cytochrome P450 in aquatic species. Aquatic Toxicology: Molecular, Biochemical, and Cellular Perspectives. D. C. Malins and G. K. Ostrander. Ann Arbor, Lewis**:** 87 - 206.

Van den Belt, K., P. Berckmans, et al. (2004). "Comparative study on the in vitro in vivo estrogenic potencies of 17 beta-estradiol, estrone, 17 alpha-ethynylestradiol and nonylphenol." Aquatic Toxicology **66**(2): 183-195.

Vilanova, R. M., P. Fernandez, et al. (2001). "Polycyclic aromatic hydrocarbons in remote mountain lake waters." Water Research **35**(16): 3916-3926.

Westbom, R., L. Thorneby, et al. (2004). "Development of a solid phase extraction method for the determination of polychlorinated biphenyls in water." Journal of Chromatography A **1033**: 1-8.

Table 1. Water Quality measurements for Penobscot River water before (River Water) and after (Bowdoin Point, Marsh River, Marsh River 03) sediment resuspension. NA = not analyzed

Water	ANC (μe/L)	Conductivity (μS/cm)	pH	Salinity (o/oo)	DOC (mg/L)
River Water	218.96	42.36	7.35	0	8.23
Bowdoin Point	556.13	754	7.34	0	NA
Marsh River	376.5	495.5	7.40	0	25.0
Marsh River 03	434.27	906	7.33	0	NA

Table 2. Analytes and ions monitored for quantitation

Analytes	Quantification[1] Ions m/z[2]	Confirmation[2] Ions m/z	Detection limit[4] ng/L
Clb_1	188	152, 190	10
2ClB_5	222	152, 224	10
3ClB_31	256	258, 186	10
4Cl_44	292	290, 220	10
4Cl_52	292	255, 226	10
4Cl_66	292	290, 220	10
OP_DDE	318	246, 248	10
5ClB_87	326	324, 254	10
5ClB_101	326	291, 256	10
PP_DDE	318	246, 176	10
5ClB_110	326	253, 184	10
OP_DDD	235	165, 199	10
6ClB_138	360	325, 290	10
PP_DDD	235	237, 165	10
6ClB_141	360	362, 290	10
6ClB_151	360	325, 290	10
6Cl_153	360	325, 290	10
7ClB_170	394	359, 324	10
7ClB_180	394	359, 324	10
7ClB_183	394	360, 323	10
7ClB_185	394	360, 323	10
9ClB_206	464	428, 392	10
Naphthalene	128	127, 129	10
2-methylnaphthalene	142	141, 115	10
acenaphthylene	152	151, 153	10
acenaphthene	154	153, 152	10
fluorene	166	165, 167	10
phenanthrene	178	176, 179	10
anthracene	178	176, 179	10
fluoranthene	202	200, 203	10
pyrene	202	200, 203	10
benz[a]anthracene	228	226, 227	10
chrysene	228	226, 227	10
benzo[b]fluoranthene	252	250, 253	10
benzo[k]fluoranthene	252	250, 253	10

Table 2 continued

benzo[a]pyrene	252	250, 253	10
indeno[1,2,3,c,d]pyrene	276	274, 277	10
dibenz[a,h]anthracene	278	279, 279	10
benzo[g,h,i]perylene	276	274, 277	10
d10 phenanthrene (Internal Standard)	188	189, 184	
CB-143 (PCB surrogate standard))	360	325, 290	
o-terphenyl (PAH surrogate standard)	230	229, 215	

[1]Quantification ion used for quantifying each analyte
[2]Mz=mass:charge ratio
[3]Confirmation ions must be present in a specified relative abundance to positively identify the analyte of interest
[4]Detection limit was determined by spiking river water with each analyte and determining the concentration that produced a signal three times above the signal:noise ratio. The detection limit was conservatively chosen to be the same for all analytes, even though some compounds could be detected at concentrations 4 – 10 times lower.

Table 3. Instrumental conditions for analysis of organic compounds by the Hewlett Packard 6890/5973 GC/MS System

Injection conditions:	splitless injection, injection port Temp = 275°C
GC column	Phenomenex ZB-5 MS (5% phenyl methyl siloxane), 30 m length, 0.25 mm ID, 0.25mm film thickness
Temperature program:	80°C, hold for 2 minutes 80°C - 120°C @ 30°C/minute 120°C - 150°C @ 10°C/minute 150°C - 290°C @ 3°C/minute, 10 minute hold
MS parameters	electron impact ionization, 70eV [1]SIM mode

[1]SIM = selected ion monitoring

Table 4. Comparison of PCB and organochlorine recoveries from Matrix spikes using Empore discs and liquid-liquid extractions

	Empore disc		**Liquid-liquid**	
Compound	Mean[1]	Stdev2	Mean[1]	Stdev[2]
Clb[3]_1	249	21.9	89.2	20.5
2ClB_5	223	65.2	97.7	18.7
3ClB_31	155	41.1	91.7	5.0
4Cl_44	135	23.3	89.7	0.2
4Cl_52	145	20.6	95.7	1.8
4Cl_66	122	12.4	101.2	4.2
5ClB_87	93	6.9	94.5	4.3
5ClB_101	95	5.7	95.8	2.4
PP_DDE	85	4.2	97.7	3.0
5ClB_110	98	8.6	92.6	4.5
6ClB_138	61	1.8	94.8	2.1
PP_DDD	124	13.2	106.5	7.9
6ClB_141	50	7.2	96.2	1.4
6ClB_151	53	6.6	97.2	3.1
6Cl_153	54	6.1	99.1	1.3
7ClB_170	34	4.0	100.1	2.3
7ClB_180	34	5.6	97.3	2.3
7ClB_183	28	8.0	100.5	1.1
7ClB_185	28	6.0	105.4	2.2
9ClB_206	14	8.6	102.5	3.6

[1]Mean of three samples.
[2]Standard deviation
[3]Clb-# =chlorobiphenyl-IUPAC, using the naming convention of Ballschmitter and Zell (Ballschmitter and Zell 1980).
[4]RCl_44=chlorobiphenyl congener 44. The number preceding the Cl indicates the degree of chlorination (here, 4 chlorine atoms). The number following the Cl is the IUPAC designation (Ballschmitter and Zell 1980).

Table 5. Comparison of PAH recoveries from matrix spike samples extracted by empore disc and liquid-liquid extraction

	Empore disc		**Liquid-liquid**	
Compound	Mean[1]	Stdev2	Mean[1]	Stdev[2]
Naphthalene	61.9	7.4	88.7	20.9
2-methylnaphthalene	68.3	7.2	95.5	16.8
acenaphthylene	50.5	9.8	111.8	21.6
acenaphthene	80.1	9.3	111.3	15.5
fluorene	87.4	10.6	127.0	17.1
phenanthrene	82.6	7.4	126.4	18.3
anthracene	41.8	5.8	114.9	12.7
fluoranthene	65.6	1.3	112.1	9.1
pyrene	66.2	1.4	107.0	7.5
benz[a]anthracene	2.5	0.1	74.9	6.1
chrysene	3.6	0.6	80.9	5.8
benzo[b]fluoranthene	1.4	0.3	56.6	2.0
benzo[k]fluoranthene	1.6	0.5	62.9	3.3
benzo[a]pyrene	0.0	0.0	49.1	0.2
indeno[1,2,3,c,d]pyrene	0.0	0.0	48.7	11.1
dibenz[a,h]anthracene	0.0	0.0	72.5	8.6
benzo[g,h,i]perylene	0.1	0.1	60.0	3.6

[1]Mean of threee samples. [2]stdev = standard deviation

Table 6. Water quality measurements for fathead minnow rearing and spawning water at the Aquaculture Research Center

Parameter	
Temperature (C)	25
pH	7 - 8
Alkalinity (ppm $CaCO_3$)	100
Hardness (ppm $CaCO_3$)	120
Flow rate (mL/min)	160
Dissolved oxygen (mg/L)	6.5
Total Ammonia (ppm NH_3-N)	<0.25
Nitrate (ppm NO_3^{-2})	1 – 2
Nitrate (ppm NO^{-2})	0

Table 7. Primer sequences for zebrafish messenger RNA quantification by Q-PCR. See methods for details.

Gene product	**5' forward primer**	**5' reverse primer**
cyp1a1	CCTGGGCGGTTGTCTATCTA	TGAGGAATGGTGAAGGGAAG
vitellogenin 1	TTTGAACGAGCAACGAACAG	AGTTCCGTCTGGATTGATGG
18s rRNA	CATGGCCGTTCTTAGTTGGT	CGGACATCTAAGGGCATCAC

Table 8. Total mercury concentrations in Penobscot River water before (River Water) and after (Bowdoin Pt, Marsh River, Marsh River 03) resuspension of Penobscot River sediments. All values in ng/L (ppt). Replicate injections were within 0.6%.

Water Sample	Total Mercury (ng/L)
River Water	3.29
Bowdoin Point	22.5
Marsh River	82.6
Marsh River 03	61.7
Method Blank	0.05
Filter Blank	1.13

Table 9A. Metal concentrations in Penobscot River water before (River Water) and after (Bowdoin Pt, Marsh River, Marsh River 03) resuspension of Penobscot River sediments. All values in ng/L (ppt). SRM = standard reference material. Duplicate injections were all within 5% except for Cd and Al.

Metal Water Sample	Cd (ng/L)	Cs (ng/L)	Ba (ng/L)	Pb (ng/L)	U (ng/L)	Ag (ng/L)	Al (ng/L)
River Water	11	3	19821	73	93	5	63966
Bowdoin Pt	17	17	23219	340	152	18	49599
Marsh River	27	19	30068	429	155	24	87303
Marsh River 03	34	21	54701	1046	89	36	96033
Lab Blank	0.08	0.03	0.89	0.22	2.22	0.06	142.57
SRMcheck	14	7	12483	79	49	4	53625
duplicate inj w/in (%):	10.6%	5.7%	1.7%	1.5%	3.7%	0.0%	28.7%

Table 9B. Metal concentrations in Penobscot River water before (River Water) and after (Bowdoin Pt, Marsh River, Marsh River 03) resuspension of Penobscot River sediments. All values in ng/L (ppt). SRM = standard reference material. Duplicate injections were within 3%.

Metal Water Sample	V (ng/L)	Cr (ng/L)	Mn (ng/L)	Co (ng/L)	Ni (ng/L)	Cu (ng/L)	Zn (ng/L)
River Water	226	228	1914	31	1146	5860	9270
Bowdoin Pt	4136	764	4742	83	1724	5819	13689
Marsh River	4740	805	2107	68	1936	6720	18024
Marsh River 03	1852	753	18672	84	2551	6020	18140
Method Blank	0.78	8.63	9.35	0.40	1.86	1.58	7.13
SRMcheck	346	320	3536	34	682	1696	913
duplicate inj w/in (%):	2.2%	1.7%	0.0%	3.0%	1.8%	1.6%	3.3%

Table 10. Organic analyte concentrations in filtered river water before and after sediment resuspension

Analytes	River water ng/L	Bowdoin Point ng/L	Marsh River ng/L	Marsh River 03 ng/L
Naphthalene	19.8 ± 11.4[1]	45.8 ± 15.1	18.3 ± 8.2	41.0 ± 10.0
2-Methylnaphthalene	2.84 ± 1.3	69.9 ± 0.5	9.4 ± 13.3	70.1 ± 4.8
Phenanthrene	2.72 ± 1.5	21.2 ± 11.5	39.4 ± 29.3	16.9 ± 8.1
Fluoranthene	2.13 ± 1.3	42.3 ± 23.0	74.0 ± 14.0	39.4 ± 24.2
Pyrene	1.80 ± 1.1	40.2 ± 26.1	99.8 ± 26.5	11.7 ± 15.7
Σ PAH	26	150	232	109

[1]Mean +/- SD for 2 replicate analyses

Table 11. Development parameters for fathead minnow embryo-larvae exposed to graded doses of the PAH toxicant, β-naphthoflavone (βNF).

Treatment (μM βNF)	N[a]	Percent Hatch	Percent Survival	Percent Deformities, type	Mean day to reach L2
Culture water[b]	6	100	99.2 (1.9) [c]	5.8 (4.4), PE[d]	8.2 (0.3)
DMSO (0.01%)		100	98.7 (2.3)	8.2 (6.8), PE	7.8 (0.1)
0.0001	3	100	97.0 (5.2)	4.2 (4.0), PE	8.0 (0.1)
0.01	3	100	93.7 (7.3)	10.5 (3.4), PE	7.9 (0.3)
0.1	3	100	96.6 (3.0)	6.8 (2.8), PE	8.4 (0.2)
1.0	3	100	94.7 (9.2)	43.4 (1.0), PE,JM,YS,GA	8.3 (0.2)

[a]number of replicate experiments (each expt: 3 pools, 25 embryos/pool)
[b]water from the Aquaculture Research Center in which adult fathead minnows were maintained and spawned.
[c]Mean (SD)
[d]PE-pericaridal edema, JM- jaw malformations, YS- yolk sac edema, GA-gill abnormalities

Table 12. Development parameters for fathead minnow embryo-larvae exposed to Penobscot River water before (River water) and after (Bowdoin Point, Marsh River, Marsh River 03) resuspension with river sediments. Set #1 – March/April 2006.

Treatment	N[a]	Percent Hatch	Percent Survival	Percent Deformities, type	Mean day to reach L2
Culture water[b]	4	100	100	2.0 (2.3), PE[d]	7.7 (0.1)
River water	4	100	99.0 (2.0)	2.0 (2.3), PE	7.3 (0.1)*
Bowdoin Point	4	100	98.0 (2.3)	4.0 (5.7), PE	7.8 (0.2)#
Marsh River	4	94.0 (9.5)[c]	80.0 (20.4)	4.0 (3.3), PE	8.1 (0.2)#
Marsh River03	4	100	99.0 (2.0)	4.0 (3.3), PE	8.1 (0.2)#

[a]number of replicate experiments (each expt: 5 pools, 5 embryos/pool)
[b]water from the Aquaculture Research Center in which adult fathead minnows were maintained and spawned.
[c]Mean (SD)
[d]PE-pericardial edema
*significantly different from Culture water @ $p<0.01$
#significantly different from River water @ $p<0.001$

Table 13. Development parameters for fathead minnow embryo-larvae exposed to Penobscot River water before (River water) and after (Bowdoin Point, Marsh River, Marsh River 03) resuspension with river sediments. Set #2 – May 2006.

	N[a]	Percent Hatch	Percent Survival	Mean day to reach L2
Culture water[b]	3	87.8 (7.1)[b]	85.1 (10.1)	8.1 (0.4)
River water	3	89.3 (2.3)	74.7 (10.1)	8.0 (0.6)
Bowdoin Point	3	90.7 (10.1)	84.0 (6.9)	7.9 (0.5)
Marsh River	3	89.3 (10.1)	78.7 (16.2)	7.9 (0.2)
Marsh River 03	3	94.7 (9.2)	89.3 (8.3)	7.8 (0.2)

[a]number of replicate experiments (each expt: 5 pools, 5 embryos/pool)
[b]water from the Aquaculture Research Center in which adult fathead minnows were maintained and spawned.
[c]Mean (SD)

Figure Legends

Figure 1. Normal and abnormal development in fathead minnows. (a, b) appearance of heart, yolk sac, scales, jaw and gill analgens in normally developing fathead minnows, stages 28 – L2, (c-f) βNF-induced (c) abnormalities in jaw and scales, (d) abnormal jaw, yolk sac edema, pericardial edema, (e) peridcardial edema, (f) gill anlagen and jaw abnormalities.

Figure 2. Innate immune system function, measured by respiratory burst response, of zebrafish embryo-larvae exposed for 5 days to Penobscot River water before (river water) and after (Bowdoin Point, Marsh River, Marsh River 03) sediment resuspension. Values represent mean +/- SEM, n=5 replicate experiments of 6 embryos each. Significantly different from RW at *P<0.003, **P<0.001. CW = culture water, RW=river water, BP=Bowdoin Point, MR=Marsh River, MR03=Marsh River 2003 collection.

Figure 3. Vitellogenin gene expression (mRNA levels) in zebrafish embryos exposed to Penobscot River water before (River water) and after (Bowdoin Point, Marsh River, Marsh River 03) resuspension with river sediments. Values represent means +/ - exponential error of the mean for 3 intrassay replicates. Significantly different from *laboratory control water, # river water, + Marsh River

Figure 4. Estrogenic response measured using MVLN cells exposed to Penobscot River water before (River water) and after (Bowdoin Point, Marsh River, Marsh River 03) resuspension with river sediments. Media treatment served as negative control, 17β-estradiol (17β-E2) served as positive control, and ICI was used to confirm chemical specificity for the estrogen receptor. Values represent means +/ SEM, for 3 replicate wells per treatment. *Significantly different from all other treatments at P<0.05.

Figure 5. CYP1A gene expression (mRNA levels) in zebrafish embryos exposed to Penobscot River water before (River water) and after (Bowdoin Point, Marsh River, Marsh River 03) resuspension with river sediments. CW= culture water. Values represent means +/ SEM for 3 intrassay replicates. Significantly different from *laboratory control water, #river water.

Figure 6. Response of zebrafish embryos carrying a transgene for metallothionein to exposure to Penobscot River water before (River water) and after (Bowdoin Point, Marsh River, Marsh River 03) resuspension with river sediments. Values represent means +/ SEM for 3 replicate wells per treatment.

Figure 1.

a, c, e

b, d, f

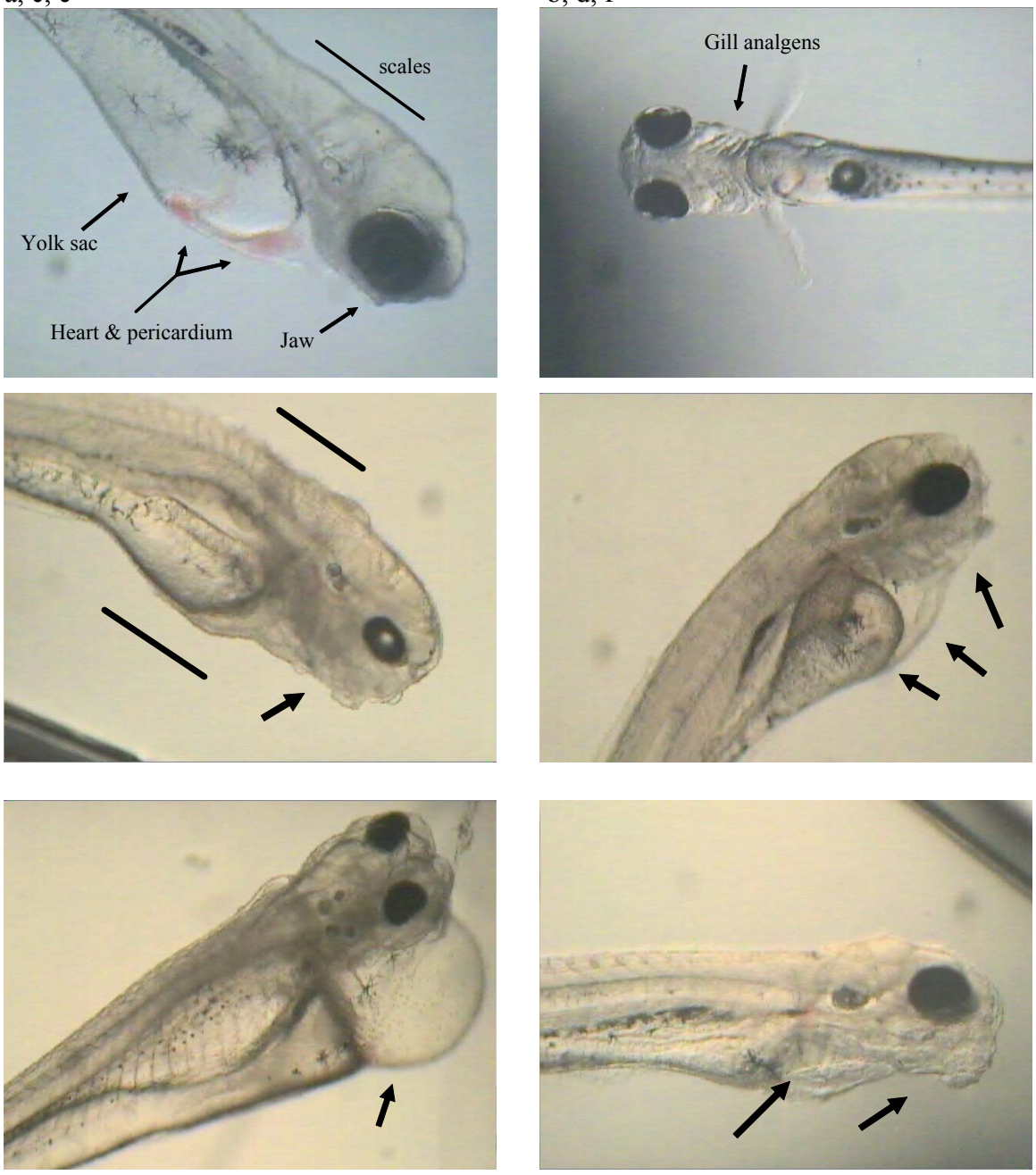


Figure 2.

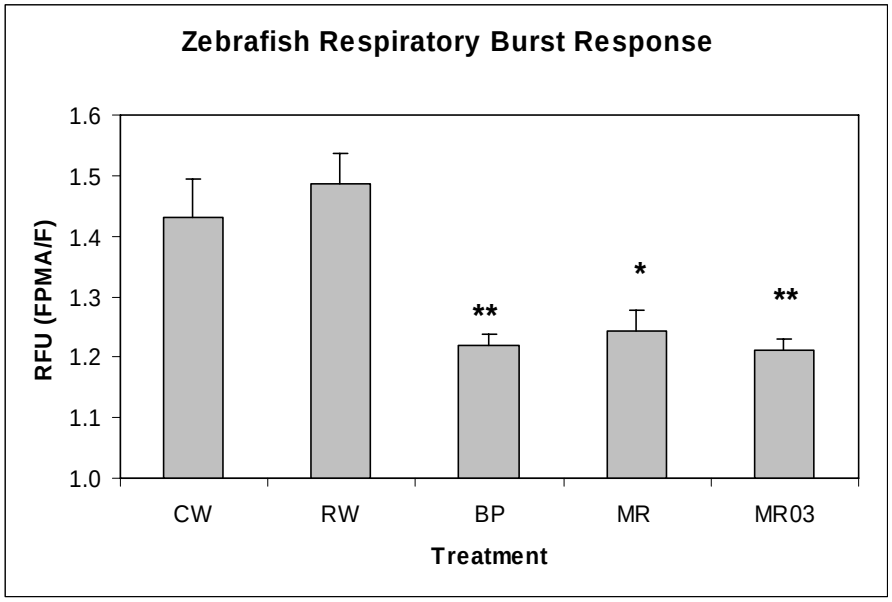

Zebrafish Respiratory Burst Response
RFU (FPMA/F)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
**
*
**
CW
RW
BP
MR
MR03
Treatment

Figure 3

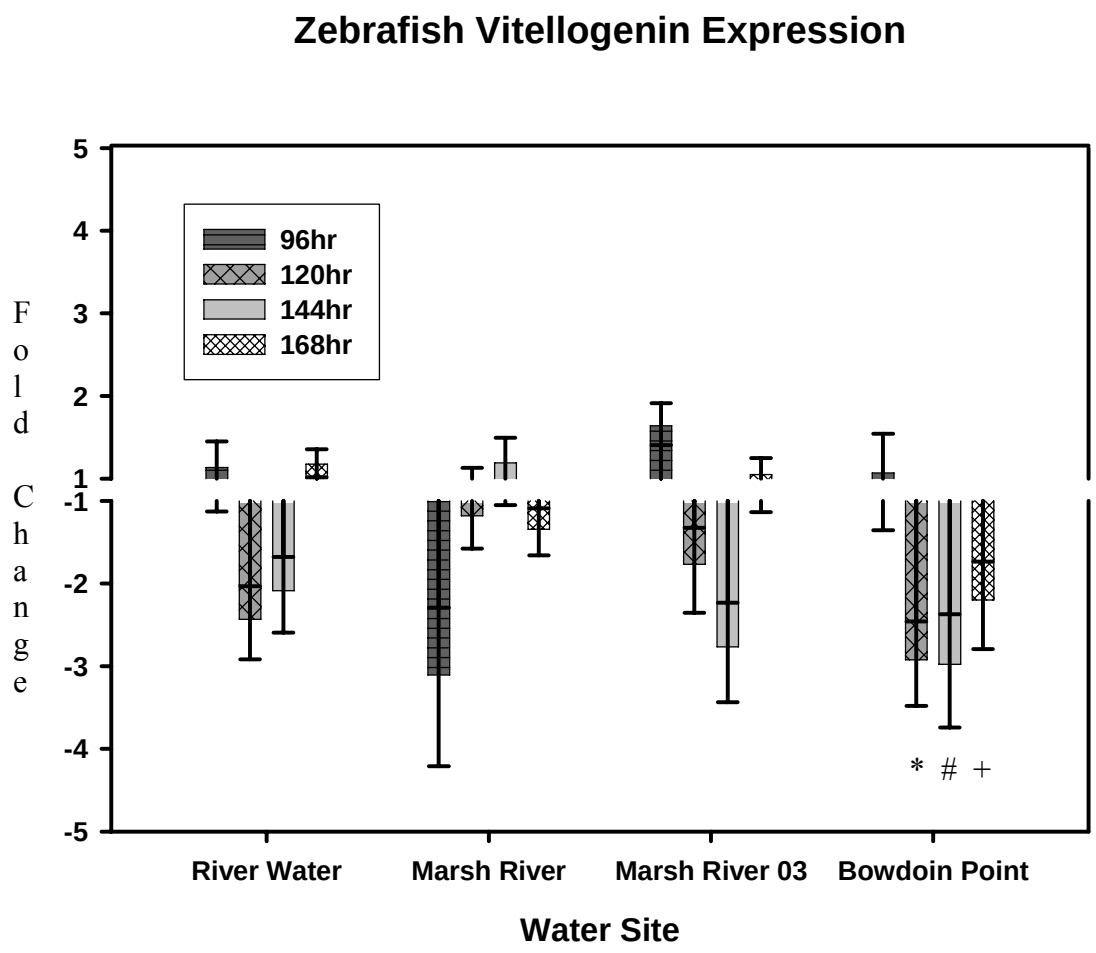

Zebrafish Vitellogenin Expression
Fold Change
5
4
3
2
1
-1
-2
-3
-4
-5
96hr
120hr
144hr
168hr
* # +
River Water
Marsh River
Marsh River 03
Bowdoin Point
Water Site

Figure 4.

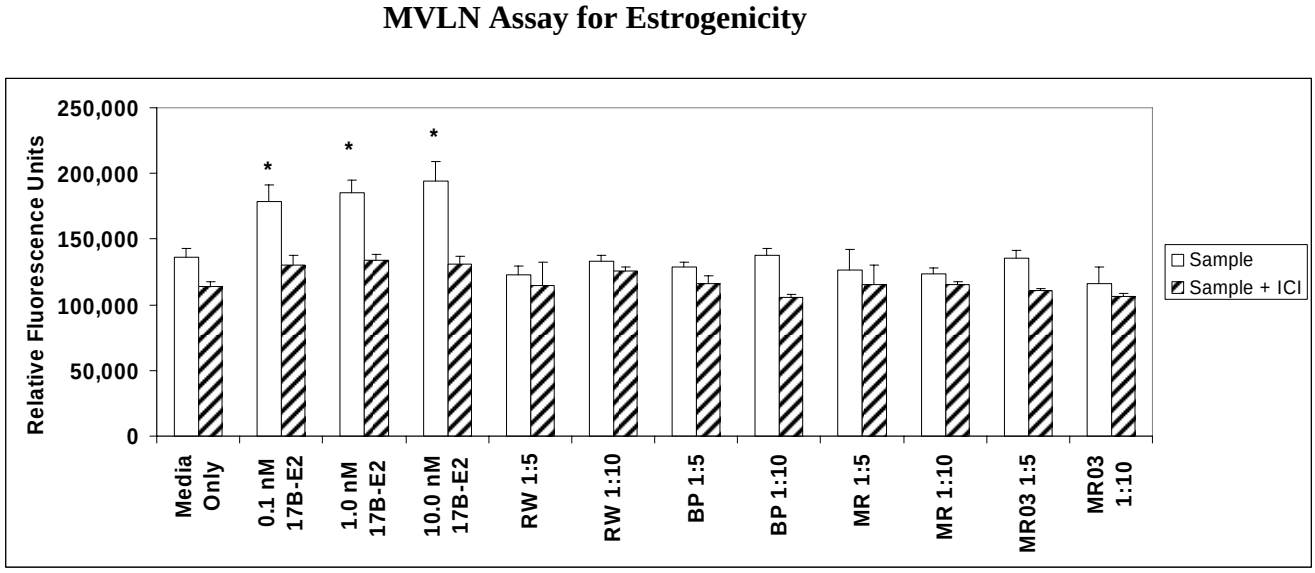

MVLN Assay for Estrogenicity
Relative Fluorescence Units
250,000
200,000
150,000
100,000
50,000
0
*
*
*
Media Only
0.1 nM 17B-E2
1.0 nM 17B-E2
10.0 nM 17B-E2
RW 1:5
RW 1:10
BP 1:5
BP 1:10
MR 1:5
MR 1:10
MR03 1:5
MR03 1:10
Sample
Sample + ICI

Figure 5

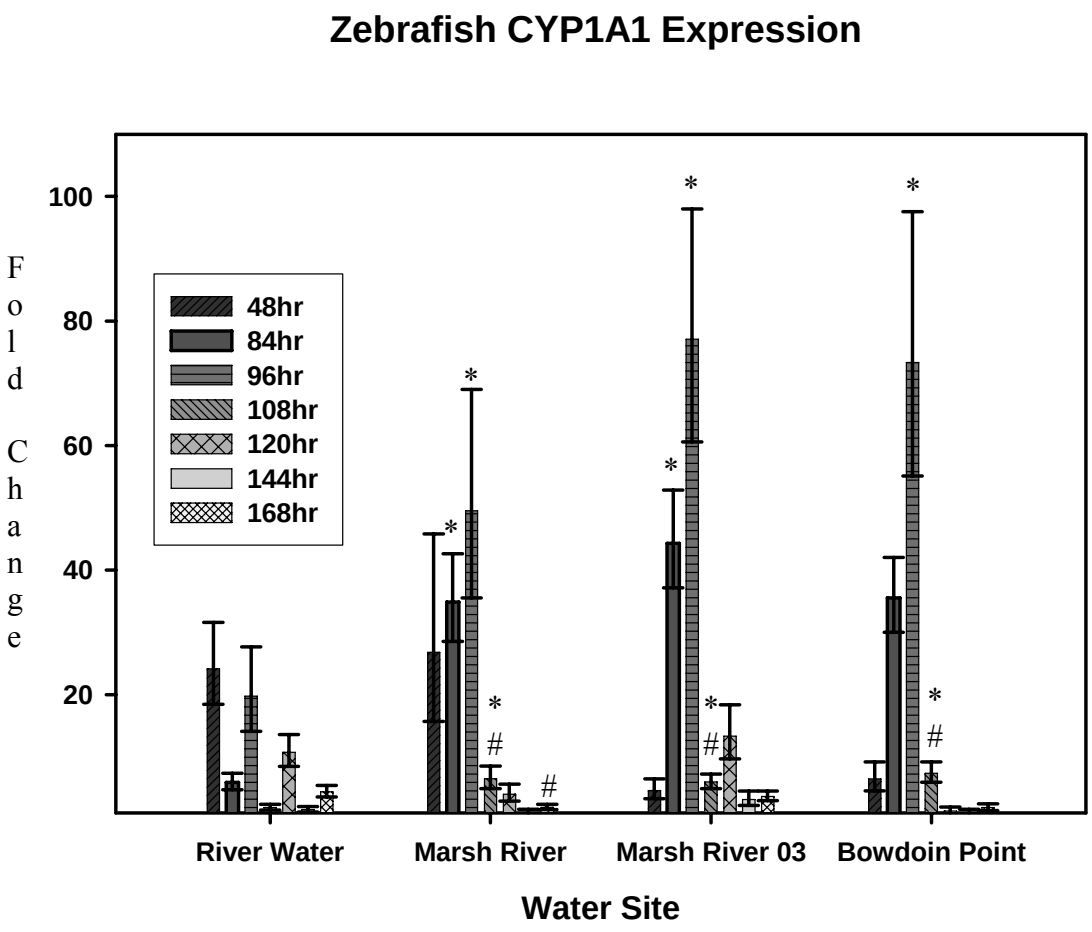
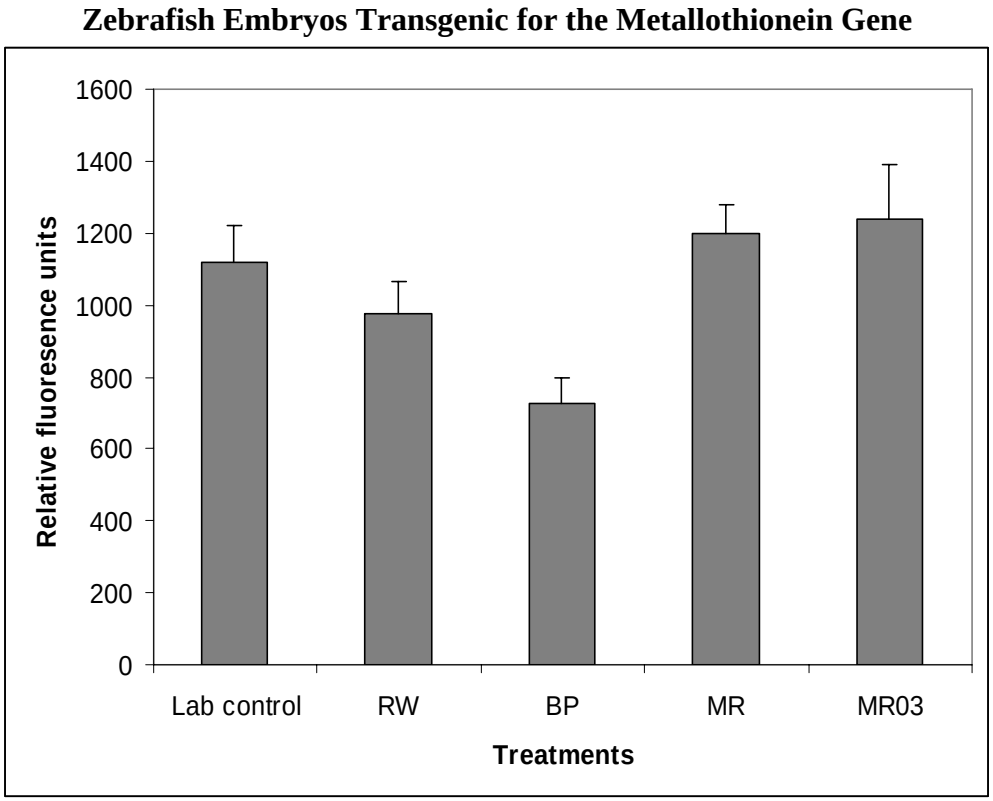

Zebrafish CYP1A1 Expression
Fold Change
100
80
60
40
20
48hr
84hr
96hr
108hr
120hr
144hr
168hr
River Water
Marsh River
Marsh River 03
Bowdoin Point
Water Site

Figure 6.


Zebrafish Embryos Transgenic for the Metallothionein Gene
Relative fluoresence units
1600
1400
1200
1000
800
600
400
200
0
Lab control
RW
BP
MR
MR03
Treatments